

# A study of energy minimization techniques applied to protein design

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(February 1, 2008)

We present a detailed study of the performance and reliability of design procedures based on energy minimization. The analysis is carried out for model proteins where exact results can be obtained through exhaustive enumeration. The efficiency of design techniques is assessed as a function of protein lengths and number of classes into which amino acids are coarse grained. It turns out that, while energy minimization strategies can identify correct solutions in most circumstances, it may be impossible for numerical implementations of design algorithms to meet the efficiency required to yield correct solutions in realistic contexts. We also investigated how the design efficiency varies when putative solutions are required to obey some external constraints and found that a restriction of the sequence space impairs the design performance rather than boosting it. Finally some alternate design strategies based on a correct treatment of the free energy are discussed. These are shown to be significantly more efficient than energy-based methods while requiring nearly the same CPU time.

## I. INTRODUCTION

A few decades ago C. Anfinsen [1] showed that the structural information of naturally occurring proteins is entirely encoded by the corresponding amino acid sequence. Since then many biologists, chemists and physicists have spent their efforts in trying to identify and simulate the mechanisms through which a given sequence reaches its stable, native conformation (protein folding) [3,5,8]. The inverse problem, also known as protein design, has similarly resisted the efforts of an ever-growing number of researchers who are now tackling it with an arsenal of techniques including *ab initio* molecular dynamics and concepts of theoretical physics [2,7,11,14,17,21–27]. The complexity of the problem is enormous because, in principle, it entails an exhaustive comparison of the native states of all sequences in search for the one(s) matching the desired target structure [7,21,25].

This problem has been recently formulated into a general mathematical form appropriate for numerical implementation [25] which shows that solving the design problem for a structure,  $\Gamma$ , amounts to the identification of the amino acid sequence,  $S$ , that maximizes the occupation probability,  $P_\Gamma(S)$ ,

$$P_\Gamma(S) \equiv \frac{e^{-\beta E_\Gamma(S)}}{\sum_{\Gamma'} e^{-\beta E_{\Gamma'}(S)}} = e^{-\beta \{E_\Gamma(S) - F(S)\}} \quad (1.1)$$

where  $\beta$  is the Boltzmann factor,  $E_\Gamma(S)$  is the energy of the sequence  $S$  over the structure  $\Gamma$  and the sum in the denominator is taken over all possible structures,  $\{\Gamma'\}$  having the same length of  $\Gamma$ . The winning sequence will maximize  $P_\Gamma(S)$  at all temperatures below the folding transition temperature (where the occupation probability of the native state is macroscopic). The occupation probability  $P_\Gamma(S)$  has the following simple physical interpretation. The quantity  $F(S)$  implicitly defined in (1.1) corresponds to the free energy of sequence  $S$ . Below the folding transition the dominant contribution to  $F(S)$  comes from the ground state(s) of  $S$ . Hence, maximising the functional  $P_\Gamma(S)$  at low temperature is equivalent to identifying the sequences for which  $E_\Gamma(S)$  corresponds to the ground state energy and the ground state degeneracy is the lowest possible.

Several obstacles need to be overcome to implement Eq. (1.1). First it is necessary to know the form of  $\mathcal{H}$ , i.e. the amino acid interaction potentials. Second the calculation of  $F(S)$  for a given sequence entails, in principle, a complete exploration of the conformation space. Finally, the quantities  $E_\Gamma(S)$  need to be calculated for all sequences  $S$  in order to find the ones maximising Eq. (1.1).

The exploration of the sequence space is rather easy to carry out and may simply involve a generation of random sequences; furthermore the dimension of the sequence space is often restricted by grouping the 20 different amino acids occurring in nature into a reduced number of classes according to their chemical similarities. Instead, the sum over alternative conformations,  $\{\Gamma'\}$  in (1.1) requires the generation of physically stable structures that compete

significantly with the true native state of  $S$  to be occupied at low temperature. These give the most significant contribution to  $F(S)$  below the folding transition temperature.

This problem is usually circumvented by neglecting the free energy contribution in Eq. (1.1) [27–30]. Maximising  $P_\Gamma(S)$  then corresponds to identifying the sequence with lowest possible energy on  $\Gamma$ . This procedure is, in principle, not guaranteed to yield the correct answer. In fact, it may well be that the sequence having the smallest energy on  $\Gamma$  has an even lower energy on a different structure.

Despite the fact that the method is not rigorous it has encountered some favour due to its simplicity of use. The aim of the present paper is to investigate how efficiency/reliability of this procedure varies as

1. the number of classes into which amino acids are subdivided is increased while the peptide length is held fixed,
2. the length is changed while the number of classes is fixed,
3. the amino acids in some positions are kept quenched while the others are chosen to minimize the total energy.

These questions will be formulated in lattice contexts where an impartial and rigorous assessment of design techniques can be found with the aid of a computer. In particular we will recourse to exhaustive enumeration whenever computational resources will allow it. We will limit our structural ensemble to compact structures on a square lattice. For lengths for which exact enumeration is feasible (up to a few dozens of residues) the square lattice can yield a ratio of exposed/buried residues much closer to the real case than the cubic counterpart.

From the analysis presented here it will appear that, design techniques based on energy minimization encounter growing limitations as the number of amino acid classes and peptide length increase to approach realistic values. We will also examine approximations to the free energy that turn out to be more efficient than energy minimization procedures and take up the same amount of computational time.

Throughout this study we will adopt the following Hamiltonian

$$E_\Gamma(S) = \sum_{i,j} \Delta_{ij} \epsilon(\sigma_i, \sigma_j) \quad (1.2)$$

where  $S_i$  denotes the residue at position  $i$ ,  $\epsilon$  is the interaction matrix and  $\Delta_{ij}$  is equal to 1 if  $i$  and  $j$  are neighbouring residues that are not consecutive along the chain and zero otherwise.

## II. DESIGN BY ENERGY MINIMIZATION

Design techniques by energy minimization were first introduced by E. I. Shakhnovich in the context of lattice models [27]. This procedure has been justified within the approximation of the discrete random energy model [6] in reference [9]. One of the most stringent tests of this method was carried out in a competition between the research teams of Harvard and San Francisco [31]. The goal was to design 10 three-dimensional compact structures of 48 beads within the HP framework. The HP model consists of only two classes of amino acids, hydrophobic [H] and polar [P]. A favourable contact energy,  $\epsilon_{HH} = -1$  was assigned to two non-consecutive residues which are one lattice spacing apart, while the other interactions,  $\epsilon_{PH}$ ,  $\epsilon_{HP}$ ,  $\epsilon_{PP}$  were set equal to zero. These values are defined modulus a sufficiently negative additive constant to guarantee that the ground states are all compact. This model favours the collapse of a hydrophobic core and is thought to mimic the main driving force of protein folding [4,10,12].

The design strategy followed by the Harvard group was to consider only sequences with the same number of H and P residues (equal composition) and then identify, among them, the sequences having minimum energy on each target structure. Disappointingly a correct answer was found in only one of ten cases [31].

Without the restriction that solutions have equal number of H and P residues,  $n_H = n_P = 24$ , the energy minimization procedure would have yielded sequences with large values of  $n_H$  (so that a couple of  $H$  residues was present in correspondence of each geometrical contact of the target structure). These solutions, like their counterpart where all residues are assigned as polar, correspond to trivial answers to the design problem since they have an enormous ground state degeneracy.

A correct solution to all 10 Harvard-San Francisco problems was found recently by a careful treatment of the free energy in (1.1) [16]. The study also showed that, to a good extent, the free energy of sequences with the same composition is approximately constant. In this case, for a given composition, maximising (1.1) corresponds to minimising the energy. Therefore, provided that solutions exist at a given composition, energy minimization techniques may be apt to find them. One question that arises naturally is: which fraction of solutions having a given concentration can be found using energy minimization techniques?

## A. Two classes of amino acids.

We will answer this and other questions by considering first the case where the amino acids are subdivided into two classes. Two different interaction matrices will be used in order to identify the qualitative features of the energy minimization procedures that do not depend on the details of the model. The first choice of parameters corresponds to the standard HP model [12] while, for the second one, an interaction matrix previously adopted by the NEC group will be used [13].

In order to collect good statistics it was decided to perform the design study on the most encodable compact structures, i.e. compact structures that are designed by the highest number of sequences [13]. Such structures have been shown to display a high degree of geometrical regularity mimicking that found in real proteins. As a by-product of our analysis we found that the encodability property is robust against changes of the model like energy interactions or number of amino acid classes and confirm that the property of encodability has mainly a geometrical origin [13,18]. For example we found that the most designable structures of length 16 and 25 (see Fig. 1) remained the same when using the the HP parameters or the NEC ones.

The main tool used for the analysis was a double backtracking algorithm, which mounted every sequence of length  $L = 16$  with  $n_H = 8$  (they are nearly 13000) on each of the 69 compact conformations. Working at fixed concentration, we then calculated the number,  $n_\Gamma(E)$ , of sequences that admitted  $\Gamma$  as their unique ground state and have energy  $E$ , as well as the total number of sequences,  $N_\Gamma(E)$ , which attain an energy  $E$  when mounted on  $\Gamma$ , irrespective of what their ground state is.

The behaviour of  $n_\Gamma(E)$  is shown in the upper plot of Fig. 2, while in Fig. 2b) is sketched the ratio  $n_\Gamma(E)/N_\Gamma(E)$ . Interestingly  $n_\Gamma(E)$  has the shape of a bell and shows that only a small fraction of solutions, 3 out of 100, have minimum energy  $E = -6$  since the overall number of sequences having minimum energy is four so the fraction of them which are a correct solution to the problem is 0.75.

In Fig. 3 we have represented analogous results for the  $L = 25$  case. The sequences were constrained to have  $n_H = 16$ ; there are approximately  $10^6$  such sequences, while the number of compact structures is 1081. Analogous enumerations were carried out for the same sequence lengths and compositions but adopting the NEC interaction parameters,  $\epsilon_{HH} = -2.3$ ,  $\epsilon_{PH} = \epsilon_{HP} = -1.0$ ,  $\epsilon_{PP} = 0$ . The results were qualitatively similar to those of the pure HP model, a part from the fact that the overall number of design solutions was greatly enhanced. For these reasons we present only the results for length 25 and  $n_H = 16$ , as shown in Fig. 4. It can be seen that  $n_\Gamma(E)$  presents an oscillatory behaviour. This is due to the fact that there is a small number of amino acid classes and that the entries of the interaction matrix have similar strength. In fact, two closely spaced energy levels could be obtained using very different sets of contact pairs; the oscillations of  $n_\Gamma(E)$  reflect the fact that the number of sequences contributing to the two sets of contacts may vary significantly.

## B. Three and four classes of amino acids.

Finally the case where the amino acids are subdivided in 3 and 4 classes was addressed. It is often remarked that the shortcomings of energy minimization routines observed in HP-like contexts are due to the artificially large ground state degeneracy [31]. Introducing more classes of amino acids will typically remove this artifact and may possibly lead to an improved performance of energy-minimization schemes.

Due to the large increase of sequence-space volume an exhaustive enumeration is feasible only for chains of length 16. Our interaction matrix for the 3 classes case was the following,

$$\begin{pmatrix} 0 & -0.5 & -1.2 \\ -0.5 & -1.15 & -1.7 \\ -1.2 & -1.7 & -2.6 \end{pmatrix}. \quad (2.1)$$

The entries in (2.1) were chosen so that the segregation principle is satisfied. For symmetric interactions this corresponds to requiring that

$$\epsilon_{ii} + \epsilon_{jj} \leq 2\epsilon_{ij}, \quad (2.2)$$

a property that is satisfied to a large extent by extracted potentials like Miyazawa-Jernigan [19] or Maiorov-Crippen [20]. The matrix (2.1) may be regarded as an extension of the NEC one, since the submatrice corresponding to the interaction between the first two types of residues is equal, a part from a scaling factor of 1/2, to that used in reference [13].

The requirement to use a fixed concentration entails the subdivision of sequences into 153 distinct bins. The performance of design algorithms based on energy minimization is not uniform across bins; in particular, for some concentrations, the method may fail to find solutions. For two classes of amino acids this occurs, for example, for  $L = 16$ ,  $n_H = 9$  and NEC parameters, where no correct solution can be identified with the energy minimization despite the existence of 190 solutions with that length and composition. The optimal bin for the analysis were chosen so that they contained the highest number of solutions. This insures the collection of the best possible statistics on the behaviour of  $n_\Gamma(E)$  and  $n_\Gamma(E)/N_\Gamma(E)$ . For three classes, one of the most populated bins corresponded to nearly equal composition:  $n_1 = 5$ ,  $n_2 = 5$ ,  $n_3 = 6$ , where  $n_i$  denotes the number of residues of type  $i$ . The results are shown in Fig. 5.

Finally, for the 4 types case we extended the matrix (2.1) to

$$\begin{pmatrix} 0 & -0.5 & -1.2 & -1.3 \\ -0.5 & -1.15 & -1.7 & -2.0 \\ -1.2 & -1.7 & -2.6 & -2.7 \\ -1.3 & -2.0 & -2.7 & -3.0 \end{pmatrix}, \quad (2.3)$$

and chose to work with sequences with  $n_1 = 7$ ,  $n_2 = 6$ ,  $n_3 = 2$ ,  $n_4 = 1$ . At this composition there exist nearly  $6 \cdot 10^5$  solutions; the behaviour of  $n_\Gamma(E)$  and  $n_\Gamma(E)/N_\Gamma(E)$  is represented in Fig. 6.

### III. LIMITATIONS OF THE ENERGY MINIMIZATION PROCEDURE

The results presented so far show that energy minimization procedures can be effective in selecting correct ground states of model proteins of reasonable length and number of amino acids. This may come as a surprise since, in principle, the method is not guaranteed to work. Part of successes observed here were undoubtedly due to the choice of highly encodable target structures. Given that there exist many sequences that are solution to the design problem (hundreds to thousands according to length, number of classes etc.) it is plausible that a handful of them will have very low values of energy. These will be the (only) ones selected by energy minimization procedures. This interpretation is corroborated by the fact that, when dealing with target structures that are poorly encodable; (e.g. structures with 20 or fewer design solutions), no correct answer to design can be normally found through energy minimization schemes. This fact also sheds some light on the failure of the Harvard attempts to solve the Harvard-San Francisco problems. In fact, the target structures used on that occasion were chosen at random and not according to designability criteria. As argued in the original solution to the problem this was also the reason why only intermediate (degenerate) solutions could be found to all 10 Harvard-San Francisco problems.

In the rest of this section we will comment on the limitations that affect energy minimization schemes even when they are adopted in very favourable circumstances such as on designable compact structures.

The first limitation regards how much the curve  $n_\Gamma(E)/N_\Gamma(E)$  is “squeezed” against the minimum energy boundary. In optimization procedures applied to realistic off-lattice contexts where the number of classes and peptide lengths is too large to allow a thorough search of the whole sequence space, the minimization procedure will typically come close to the lowest possible energy but without reaching it. It is then paramount to examine “how close” it is necessary to get to the ground state energy to ensure that a significant fraction of the sequences having that energy is a solution to the problem. As a quantitative measure we introduce the parameter

$$x = \frac{\tilde{E} - E_{min}}{E_{max} - E_{min}} \quad (3.1)$$

where  $E_{min}$  and  $E_{max}$  are respectively the minimum and maximum energies for which solutions to the design problem exists and  $\tilde{E}$  is the energy below which a randomly picked sequences has more than 50% chance to be a solution to the design problem. Thus, the lower the value of  $x$  ( $0 < x < 1$ ) the worst is the performance of the method.

Since the curves  $n_\Gamma(E)/N_\Gamma(E)$  typically do not show a smooth behaviour,  $\tilde{E}$  is determined with the aid of a high-order polynomial function interpolating  $n_\Gamma(E)/N_\Gamma(E)$ . For the results of Fig. 2 we have  $x_{16} = 0.38$ .

Upon increasing the chain length the shoulder of the curve is shifted closer to the minimum energy edge; in fact, from Fig. 3 we have  $x_{25} = 0.28$ . Finally, we considered the most encodable structure of length 36 and considered sequences with the same length and  $n_H = 18$ . Since it is not feasible to mount all the sequences with this composition on each of the 57337 compact structures we resorted to a random sampling of  $10^7$  sequences. We obtained  $x_{36} = 0.20$ . The values of  $x_{16}$ ,  $x_{25}$  and  $x_{36}$  were found not to change appreciably when using a different composition provided that there exist a significant number of solutions.

A similar trend can be observed by increasing the number of amino acid classes. For the results of Figs. 5 and 6 one has  $x_{3c} = 0.33$  and  $x_{4c} = 0.24$  showing a steady decrease as a function of the number of classes (also remember that for two classes we had  $x_{16} = 0.4$ ).

This shows that the demand on computational efficiency grows rapidly as a function of length and classes. For realistic design on proteins with a few hundred residues and 20 types of amino acids the required efficiency may fall beyond the reach of computational techniques.

Nevertheless, even if it were possible to find the sequence(s) with minimum energy, other issues need to be addressed. In particular, a limitation having far reaching consequences is that the number of correct solutions that can be identified is only a tiny fraction of the existing ones. For example, for  $L = 16$ ,  $n_H = 8$  the fraction is 3/100 when using HP parameters and 3/207 for NEC ones. These figures drop respectively to 24/1971 and 21/3978 for length 25,  $n_H = 16$ . The proportion decreases more dramatically when considering more than two classes of amino acids, as can be seen in the plots of Fig. 5 and Fig. 6. In designing realistic off-lattice proteins this feature is likely to pose severe limitations to the reliability of the method.

In fact, it is expected that residues in naturally occurring sequences were not selected on mere energetic considerations but also on structural and biological functionality. These solutions would be correctly identified when maximising the low temperature occupation probability (1.1) which, as said before, has 100 % efficiency throughout the energy range. On the contrary they could be missed easily by energy-minimization schemes since sequences with minimum energy on a given protein backbone may not be the most suitable ones as far as biological function is concerned.

Yet energy minimization approaches could, in principle, still be salvaged by arguing that the active sites of a protein are a small fraction of the total residues and, when known, may be fixed *a priori*. In an attempt to “improve on nature” (e.g. to increase the thermodynamic stability of the native fold) the rest of the residue could be found subsequently by energy minimization.

In our lattice studies we have found considerable evidence against this picture. We considered solutions to the design problem with intermediate energy and selected a small number of residues. Then we performed an energy minimization procedure over sequences that *a)* had the same concentration as the reference sequence and *b)* were equal to the reference sequence in correspondence of the selected residues. In our attempts we found that very frequently the putative solution was wrong. This is best illustrated with a simple example given for chains of length 16 and two classes of amino acids interacting via the NEC potentials. In Fig. 7a) a ground state conformation having energy  $E = -13.2$  is shown. By quenching two residues at position 4 and 14 and minimizing the energy at constant composition one obtains the sequence HPHPPPPHPHHHPPH. The energy of this sequence on the original structure is  $E = -13.5$  but its native state is the structure shown in Fig. 7b) with energy -14.5. This example is not an exceptional case since nearly one in 8 random attempts to quench two residues of intermediate sequences and minimizing the energy failed to give good solutions. This is a significant failure rate since it must be borne in mind that nearly one half of residues occupy “hot” positions that constrain them to be of a well defined type (e.g. P residues at corners) for most solutions. When quenching two residues at cold positions the failure rate could be over 50%. Quenching three key residues in chains of length 25 may lead to a design failure rates as high as 30%. This shows that introducing more constraints on the designed sequence (besides its overall composition) impairs severely the design method instead of making it more reliable.

#### IV. SIMPLE ALTERNATIVE DESIGN STRATEGIES

While energy minimization methods appear to be unsatisfactory from both theoretical and numerical points of view, they are typically simple and fast to implement.

On the other hand, recouring to rigorous techniques that take into proper account the free energy term in Eq. (1.1) may, in proportion, require much more CPU time since each trial solution needs to be mounted on alternative conformations,  $\{\Gamma'\}$  (see 1.1). The obvious payoff is that *all* solutions to design problems can be identified with 100% success throughout the whole energy range [ 25,16]. There are, however, several design procedures based on approximate treatments of the free energy that, while having the same speed of energy minimization methods, are much more efficient [ 7,16,17,26].

These procedures were first developed in the attempt to design real proteins [ 17]. In that case, contrary to lattice models, it was impossible to generate alternative off-lattice structures competing with the target one (doing so would amount to be able to perform a direct folding). Rather it was decided to exploit the properties that  $F(S)$  formally depends only on the sequence  $S$  to expand it as a function of composition and other sequence parameters. Contrary to energy minimization techniques this method did not require any external intervention to fix the correct composition. Remarkably the correct ratio of  $n_H/n_P$  was nevertheless observed in optimal solutions [ 17].

Design strategies based on a functional approach to  $F(S)$  are not only reliable, but they may even be used to

determine the best (unknown) amino acid interactions given a two-body (or higher-order) parametrization of the Hamiltonian. Details on the use of these methods can be found elsewhere [ 17,26]; in the rest of this section we will concentrate on yet another free-energy-based design method originally proposed by Deutsch and Kurowsky [ 7]. The DK strategy is based on building a table of the relative frequency of geometrical contacts between two residues at sequence positions  $i$  and  $j$ ,  $\langle \Delta_{ij} \rangle$ , collected over all compact structures. Hence, for a given sequence,  $S$ , the free energy can be approximated as

$$F_{DK}(S) = \sum_{ij} \langle \Delta_{ij} \rangle \epsilon(S_i, S_j) . \quad (4.1)$$

This may be regarded as an average energy attained by sequence  $S$  on compact structures. Requiring that  $E(S) - F(S)$  is minimized selects the sequence(s) whose energy on  $\Gamma$  lies as low as possible with respect to  $F(S)$ . This method can be used without constraining the sequence composition to a particular value and its efficiency, albeit not equal to 100 %, is much higher than what is obtainable by energy minimization in analogous circumstances. This was assessed by ranking the sequences according to their normalized DK score:

$$\frac{2(E(S, \Gamma) - F_{DK}(S))}{|E(S, \Gamma) + F_{DK}(S)|} , \quad (4.2)$$

and isolating those that stayed below a pre-assigned threshold. For example, for the case of length 25, NEC parameters,  $8 \leq n_h \leq 14$  we chose the threshold value as 0.25. The selected sequences represented our putative solutions and were ordered according to their ground state energy. The efficiency was calculated as the ratio of correct solutions versus putative ones as a function of energy. While, at low energies, the number of correct solutions was approximately the same for the DK method and the energy minimization one, at higher ones the efficiency of the DK procedure could be 100 times higher than the energy minimization procedure. The cumulative efficiency of the DK approach compared to energy-minimization routines over the whole range of energy for which solutions existed was 20:1 (this range is easily identified with free-energy based methods, but impossible to find out with the energy minimization). The same proportion of efficiency was observed for length 36, where a random sampling of sequences was performed

## V. CONCLUSIONS

We have carried out extensive enumeration studies to assess the performance of design techniques based on energy minimization. It is found that these techniques can be effective in selecting correct ground states. Nevertheless, it turns out that the overwhelming majority of solutions do not possess minimum energy and hence cannot be identified. It was also shown that practical implementations of energy-based design strategies need to be more and more efficient in finding the lowest energy solutions on increasing the sequence length and number of amino acid classes. Finally it was found that these methods become unreliable when additional requirements are imposed on the properties of putative solutions, which possibly suggests that the method may be unsuitable to design realistic proteins with desired biological functionality.

Design techniques incorporating appropriate treatments of the free energy do not suffer these shortcomings and can, in principle, lead to 100 % design success at the expenses of considerable CPU time. In the last section of this paper we discuss some approximations to the free energy that while being as fast as energy-based methods, appear to be more efficient. Furthermore, they do not require any prior fixing of a correct amino acid concentration and could be used effectively to replace energy-minimization techniques when designing realistic proteins.

ACKNOWLEDGMENTS. We thank J. R. Banavar, R. Dima, Jort Van Mourik and R. Zecchina for useful discussions. We are indebted to Flavio Seno for illuminating suggestions and a careful reading of the manuscript.

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<sup>1</sup> Anfinsen, C., (1973), Principles that govern the folding of protein chains, *Science*, **181**, 223-239

<sup>2</sup> Bowie, J. U, Luthy, R. & Eisenberg, D., (1991), A method to identify protein sequences that fold into a known 3- dimensional structure. *Science* **253**, 164-170

<sup>3</sup> Branden, C. & Tooze, J. (1991) in *Introduction to protein structure*, Garland Publishing, New York

<sup>4</sup> Cordes, M. H. J., Davidson, A. R. & Sauer, R. T. (1996), Sequence space, folding and protein design. *Curr. Opin. in Struct. Biol.* **6**, 3-10

- <sup>5</sup> Creighton, T.E. (1983) in *Proteins: structures and molecular properties*, W. H. Freeman ed., New York
- <sup>6</sup> Derrida, B., (1981), Random-energy model - an exactly solvable model of disordered systems, *Phys. Rev. B* **24**, 2613-2626
- <sup>7</sup> Deutsch, J. M. & Kurosky, T. (1996), New algorithm for protein design. *Phys. Rev. Lett.* **76**, 323-326.
- <sup>8</sup> Fletterick, R. J., Schroer, T., & Matela, R. J. (1985) in *Molecular structure: macromolecules in three dimensions*, Blackwell Scientific, Oxford, UK
- <sup>9</sup> Gutin, A.M. and Shakhnovich, E. I. (1994), Ground state of random copolymers and the discrete random energy model, *J. Chem. Phys.* **98**, 8174-8177
- <sup>10</sup> Kamtekar, S., Schiffer, J. M., Xiong, H., Babik, J. M. & Hecht, M. H. (1993), Protein design by binary patterning of polar and nonpolar amino-acids. *Science* **262**, 1680-1685
- <sup>11</sup> Kuroda, Y., Nakai, T. & Ohkubo, T. (1994), Solution structure of a de-novo helical protein by 2d-NMR spectroscopy. *J. Mol. Biol.* **236**, 862-868
- <sup>12</sup> Lau, K. F. & Dill, K. A. (1989) A lattice statistical-mechanics model of the conformational and sequence-spaces of proteins. *Macromolecules* **22**, 3986-3997
- <sup>13</sup> Li, H., Helling, R., Tang, C., Wingreen, N. (1996) Emergence of preferred structures in a simple-model of protein folding, *Science*, 666 - 669
- <sup>14</sup> Lombardi, A., Bryson, J. W. & DeGrado W. F. (1997), De novo design of heterotrimeric coiled coils *Biopolym. Peptide Sci.* **40**, 495-504
- <sup>15</sup> Maiorov, V. N. & Crippen, M. G. (1992), Contact potential that recognizes the correct folding of globular proteins. *J. Mol. Biol.* **227**, 876-888
- <sup>16</sup> Micheletti, C., Seno, F., Maritan, A. & J. R. Banavar (1998), Protein Design in a Lattice Model of Hydrophobic and Polar Amino Acids *Phys. Rev. Lett.*, **80**, 2237-2240
- <sup>17</sup> Micheletti, C., Seno, F., Maritan, A. & J. R. Banavar (1998), Design of proteins with hydrophobic and polar amino acids *Proteins: structure function and genetics*, **32**, 80 (1998)
- <sup>18</sup> Micheletti, C., J. R. Banavar, Maritan, A., Seno, F. (1998), *Steric constraints in model proteins* *Phys. Rev. Lett.* **80**, 5683 (1998)
- <sup>19</sup> Miyazawa, S. and Jernigan R. L., (1985) Estimation of effective interresidue contact energies from protein crystal-structures - quasi-chemical approximation, *Macromolecules*, **18**, 534-552; (1996), Residue-residue potentials with a favorable contact pair term and an unfavorable high packing density term, for simulation and threading *J. Mol Biol.*, 623-644
- <sup>20</sup> V. N. Maiorov and G. M. Crippen (1992) Contact potential that recognizes the correct folding of globular- proteins *J. Mol. Biol.* **227**, 876-888
- <sup>21</sup> Morrissey, M. P. & Shakhnovich, E. I. (1996), Design of proteins with selected thermal properties *Folding and Design* **1**, 391-405
- <sup>22</sup> Pabo, C. (1983), Designing proteins and peptides. *Nature* **301**, 200
- <sup>23</sup> Ponder, J. W. & Richards, F. M. (1987), Tertiary templates for proteins - use of packing criteria in the enumeration of allowed sequences for different structural classes. *J. Mol. Biol.* **193**, 775-791
- <sup>24</sup> Quinn, T. P., Tweedy, N. B., Williams R. W., Richardson, J. S. & Richardson, D. C. (1994), Beta-doublet - de-novo design, synthesis, and characterization of a beta-sandwich protein. *Proc. Natl. Acad. Sci USA* **91**, 8747-8751
- <sup>25</sup> Seno, F., Vendruscolo, M., Maritan, A., & Banavar, J. R. (1996), Optimal protein design procedure. *Phys. Rev. Lett.* **77**, 1901-1904
- <sup>26</sup> Seno, F., Micheletti, C., Maritan, A., & Banavar, J. R. (1998), Variational approach to protein design and and extraction of interaction potentials, to be published in *Phys. Rev. Lett.* (1998).
- <sup>27</sup> Shakhnovich, E. I. (1994), Proteins with selected sequences fold into unique native conformation. *Phys. Rev. Lett.* **72**, 3907-3910
- <sup>28</sup> Shakhnovich, E. I. & Gutin A. M., (1993), A new approach to the design of stable proteins. *Protein Engineering* **6**, 793-800
- <sup>29</sup> Skorobogatiy, M., Guo, H., Zuckermann, M. J. (1997). *A deterministic approach to the protein design problem*, *Macromolecules* **30**, 3403-3410
- <sup>30</sup> Yue, K., & Dill, K. A. (1992) Inverse protein folding problem - designing polymer sequences. *Proc. Natl. Acad. Sci. USA* **89**, 4163-4167
- <sup>31</sup> Yue, K., Fiebig, K. M., Thomas, P. D., Chan, H. S., Shakhnovich, E. I., & Dill, K. A., (1995), A test of lattice protein-folding algorithms. *Proc. Natl. Acad. Sci. USA* **92**, 325-329 (1995)

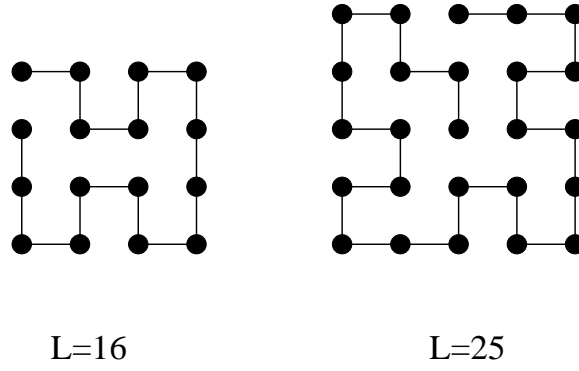


FIG. 1. The most encodable compact structures among chains of length a) 16, b) 25.

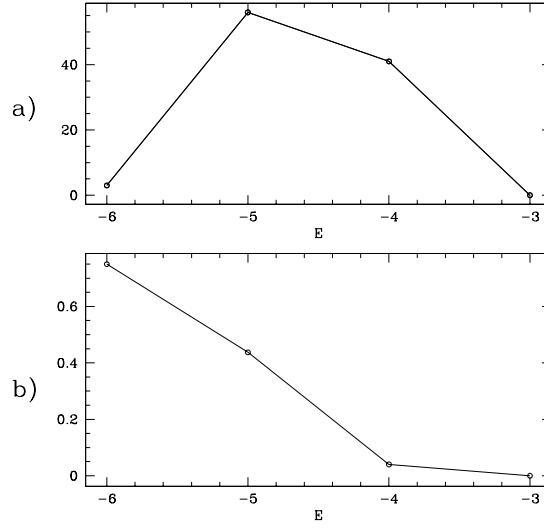


FIG. 2. Distribution of a)  $n_\Gamma(E)$ , b)  $n_\Gamma(E)/N_\Gamma(E)$  for sequences of length 16 and  $n_H = 8$  with HP-type interactions. The target structure  $\Gamma$  is shown in Fig. 1a).

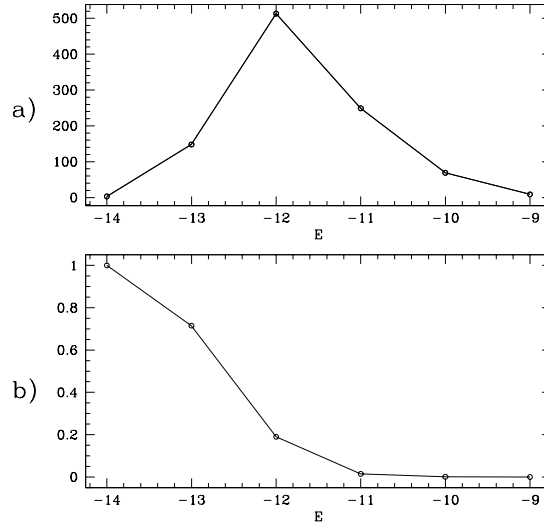




FIG. 3. Distribution of a)  $n_{\Gamma}(E)$ , b)  $n_{\Gamma}(E)/N_{\Gamma}(E)$  for sequences of length 25 and  $n_H = 16$  with HP-type interactions. The target structure  $\Gamma$  is shown in Fig. 1b)

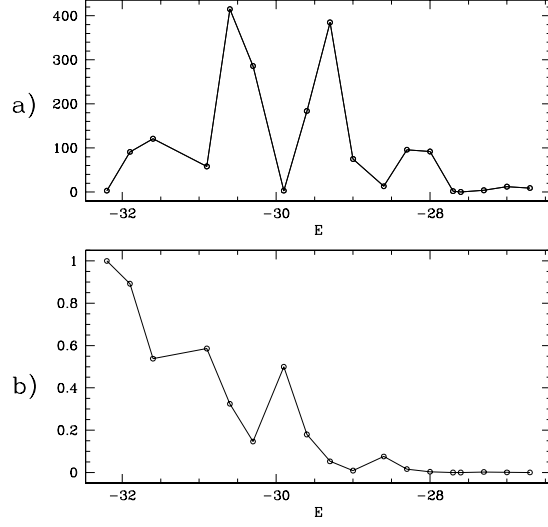


FIG. 4. Distribution of a)  $n_{\Gamma}(E)$ , b)  $n_{\Gamma}(E)/N_{\Gamma}(E)$  for sequences of length 25 and  $n_H = 16$  with NEC-type interactions. The target structure  $\Gamma$  is shown in Fig. 1b)

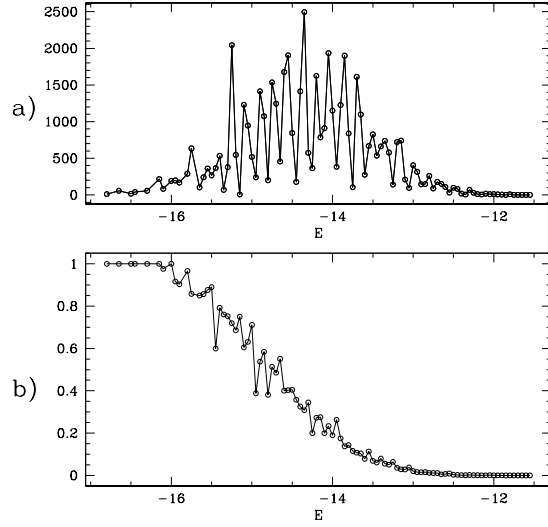


FIG. 5. Distribution of a)  $n_{\Gamma}(E)$ , b)  $n_{\Gamma}(E)/N_{\Gamma}(E)$  for sequences of length 16 and 3 classes of amino acids interacting through (2.1). The target structure  $\Gamma$  is shown in Fig. 1a).

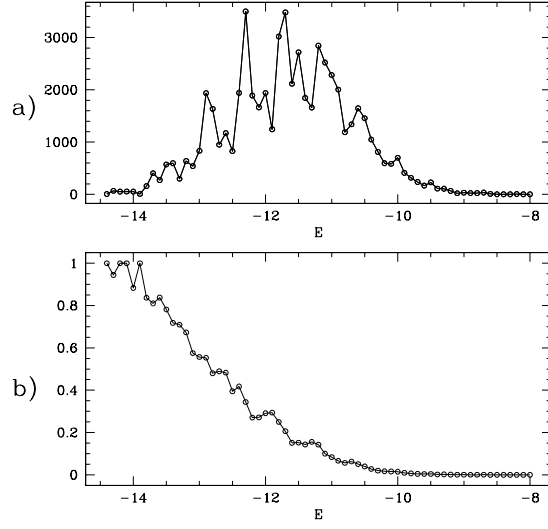


FIG. 6. Distribution of a)  $n_\Gamma(E)$ , b)  $n_\Gamma(E)/N_\Gamma(E)$  for sequences of length 16 and 3 classes of amino acids interacting through (2.1). The target structure  $\Gamma$  is shown in Fig. 1a).

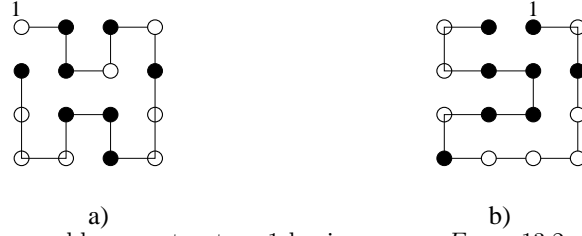


FIG. 7. a) a solution to the design problem on structure 1 having energy  $E = -13.2$ . By quenching residues 4 and 14 and minimizing the energy on the target structures one obtains sequence  $S = \text{HPHPPPPHPHHHPPH}$  having  $E_\Gamma = -13.5$ . The true ground state configuration of  $S$  has an even lower energy,  $E' = -14.5$ , as shown in b).